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Stimulation of Sterigmatocystis by chloroform

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The response of fungi to the influence of salts and other chemical compounds, as manifested by accelerated growth, increased weight, and active evolution of carbon dioxide, has been the subject of several investigations within a recent period. Raulin's * early observations have been much extended by Richards † who has ascribed ‡ the action to a more economical use of the nutrition at hand, that is, he has proved the economic coefficient of Kunstmann § to decrease as the stimulant approaches its point of optimum efficacy. Ono, || Yashuda,¶ Hattori, ** Stevens,†† Iwanoff,‡‡ Benecke§§ and others have extended the range of

* Raulin, J. Études chimiques sur la vegetation. Ann. Sc. Nat. Bot. V. 11 : 93. 1869.

† Richards, H. M. Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. 30 : 665. 1897.

‡ Richards, H. M. The effect of chemical irritation on the economic coefficient of sugar. Bull. Torrey Club 26 : 463. 1899.

§ Kunstmann, H. Ueber das Verhältniss z. Pilzernte u. verbrauchter Nährung. Inaug. Dissert. Leipzig, 1895.

|| Ono, N. Ueber die Wachsthumsbeschleunigung einiger Algen u. Pilze. Jour. Coll. Sci. Imp. Univ. Tokyo 13 : 141. 1900.

¶ Yashuda, A. On the effect of alkaloids upon some moulds. Bot. Mag. Tokyo 15. 1901.

** Hattori, H. Studien über die Einwirkung der Kupfersulfats auf einige Pflanzen. Jour. Coll. Sci. Imp. Tokyo 15 : 371. 1901.

†† Stevens, F. L. The effect of aqueous solutions upon the germination of fungus spores. Bot. Gaz. 26 : 377. 1898.

‡‡ Iwanoff, K. S. Ueber die Wirkung einiger Metallsalze und einatomiger Alkohole auf die Entwickelung von Schimmelpilzen. Bot. Centralb. 98 : 97. 1905.

§§ Benecke, W. Die zur Ernährung der Schimmelpilze nothwendigen Metalle. Jahrb. Wiss. Bot. 28 : 487. 1895 ; and Einige neuere Untersuchungen über den Einfluss von Mineralsalzen auf Organismen. Bot. Zeit. 62[2] : 113. 1904.

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toxic compounds, all with concordant results; namely, that those substances which in larger amounts are fatal, serve to stimulate the organism to increase in activity when present in sufficiently small quantities, in accordance with the generalization of Hueppe.* In addition, Ono † has found that the waste through oxalic acid formation is at the same time lessened; and Watterson ‡ that although there is greater expiration of CO_2 the increase is only parallel with that of the crop and its production relatively normal. Schulz § working with yeast; Hueppe, || Effront, ¶ and Lindet ** with bacteria; and Ono †† and Livingston ‡‡ with algae have reached like conclusions as far as growth is concerned.

Copeland and Kahlenberg, ||| and Kahlenberg and True, *** and more lately Kanda, ††† working with higher plants tell of stimulation caused by salts. Similarly Copeland ‡‡‡ has obtained increased CO_2 evolution from work with phanerogams and tadpoles, interpretating the apparent vigor, however, as an evidence of harm rather than benefit.

Townsend §§§ mixed ether with water supplied to seeds and thus hastened germination. He has also shown that seeds, subjected to HCN gas ||||| for a short time, sprout sooner and grow

* Hueppe, F. The Principles of Bacteriology, 89, 90. Trans. by E. O. Jordan.

† *L. c.*

‡ Watterson, A. The effect of chemical irritation on the respiration of fungi. Bull. Torrey Club **31**: 291. 1904.

§ Schulz. Ueber Hefegifte. Bot. Zeit. **46**: 610. 1888.

|| *L. c.*

¶ Effront. Compt. rend. **119**: 169. 1894.

** Lindet. Compt. rend. **138**: 508. 1904.

†† *L. c.*

‡‡ Livingston, B. E. Chemical stimulation of a green alga. Bull. Torrey Club **32**: 1. 1905.

||| Copeland, E. B. & Kahlenberg, L. The influence of the presence of pure metals upon plants. Trans. Wis. Acad. **12**: 454. 1899.

*** Kahlenberg, L. & True, R. H. On the toxic action of dissolved salts. Bot. Gaz. **22**: 81. 1896.

††† Kanda, M. Reizwirkung einiger Metallsalze auf das Wachsthum höherere Pflanzen. Jour. Coll. Sci. Imp. Tokyo **19**: 1. 1904.

‡‡‡ Copeland, E. B. Chemical stimulation and the evolution of CO_2. Bot. Gaz. **35**: 81. 1903.

§§§ Townsend, C. O. The effect of hydrocyanic acid gas upon grains and other seeds. Bot. Gaz. **31**: 241. 1901.

||||| Townsend, C. O. The correlation of growth under the influence of injuries. Ann. Bot. **11**: 509. 1897.

more rapidly than those not so treated; that *Avena* seedlings grown in a sufficiently weak atmosphere of ether are at first retarded and then accelerated, finally returning to their normal rate of growth if the shock be not too long continued; and that in small amounts ether vapor will aid germination in the spores of *Penicillium* and *Mucor*,* although larger doses are inimical. Duggar † says that a saturated atmosphere of chloroform is fatal to the spores of *Aspergillus flavus* and *Phycomyces* and without benefit to *Sterigmatocystis*, and that ether is of no consequence as a stimulant in germinating *Aspergillus*. With *Aspergillus* (*Sterigmatocystis*) *niger*, Kosinski ‡ finds respiration retarded when exposed to ether fumes.

Ewart,§ working with chloroform in the water of water plants noted suspension of CO_2 assimilation agreeing with the earlier results of Claude Bernard. Kegel,|| however, finds with *Elodea canadensis* an increment of CO_2 assimilation in some percentages of ether and chloroform, but a sudden check in the case of a lesser amount. On the other hand, Bonnier and Mangin ¶ were able, by adding ether carefully, to stop assimilation of CO_2 without affecting respiration, an observation in accordance with that of Schwarz,** who, with both ether and chloroform, has recorded stoppage of oxygen evolution not accompanied by elimination of CO_2 evolution until the plant was fatally affected. Ewart's †† results with mosses were similar. Movement in the protoplasm of *Philotria* (*Elodea*),

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* Townsend, C. O. Effect of ether upon the germination of seeds and spores. Bot. Gaz. 27: 458. 1899.

† Duggar, B. M. Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. 31: 38. 1901.

‡ Kosinski, I. Die Athmung bei Hungerzuständen u. unter Einwirkung von mechanischen u. chemischen Reizmitteln bei Aspergillus niger. Jahrb. Wiss. Bot. 37: 137. 1902.

§ Ewart, A. J. Action of chloroform on CO_2 assimilation. Ann. Bot. 12: 415. 1898.

|| Kegel, W. Ueber den Einfluss von Chloroform und Aether auf die Assimilation von Elodea canadensis. Inaug. Dissert. Göttingen, 1905.

¶ Bonnier, G. & Mangin, L. Recherches sur l' action chlorophyllienne séparée de la respiration. Ann. Sci. Nat. VII. 3: 5. 1886.

** Schwarz, F. Zur Kritik der Methode des Gasblasenzählens an submersen Wasserpflanzen. Untersuch. Bot. Inst. zu Tübingen, 1: 97. 1881.

†† Ewart, A. J. On assimilatory inhibition in chlorophyllous plants. Jour. Linn. Soc. 31: 364. 1896.

as seen by Farmer and Waller,* ceased under the influence of ether and chloroform, the latter proving more powerful.

With various seedlings in an ether atmosphere, Morkowine † obtained increase of respiration in the light; Bonnier and Mangin ‡ got no such effect in the dark. Puriewitsch, as quoted by Morkowine,§ concluded that in ether vapor, transformation of sugar to starch is inhibited. Jumelle || believes transpiration increased in light and decreased in dark by ether, Zaleski ¶ that proteid formation is hindered by the same substance. Pfeffer ** cites Elfving †† and Laurén ‡‡ as authorities for the observation that CO_2 formation, but not growth, is augmented in higher plants by a not too long exposure to chloroform; and finally, Johanssen §§ has proved the economic value of ether in forcing early blooming in flowering plants. The period of winter rest is shortened, rapidity of growth and total evolution of CO_2 increased.

The majority of the investigations with chloroform and ether, it will be noted, have had to do with their effect on green plants. Townsend |||| and Duggar ¶¶ to be sure, experimented with fungus spores but their work stopped with germination; and Kosinski *** was concerned only with the respiration factor. It has been the aim of the piece of work here described to compare the effect of chloroform vapor on fungus growth, as shown by increase in dry substance, with the results quoted above. Therefore *Sterigmatocystis nigra* (*Aspergillus niger*) in the main and *Penicillium glaucum*

* Farmer, J. B. & Waller, A. D. Observations on the action of anaesthetics on vegetable and animal protoplasm. Proc. Royal Soc. **63**: 213. 1898.

† Morkowine, N. Recherches sur l' influence des anesthésiques sur la respiration des plantes. Rev. Gen. de Bot. **11**: 289. 1899.

‡ Bonnier & Mangin, *l. c.*

§ *L. c.*

|| Jumelle, H. Influence des anesthésiques sur la transpiration des végétaux. Rev. Gen. de Bot. **2**: 417. 1890.

¶ Zaleski, W. Zur Aetherwirkung auf die Stoffumwandlung in den Pflanzen. Ber. Deuts. Bot. Gesells. **18**: 292. 1900.

** Pfeffer. Physiology of Plants, **1**: 564. Trans. by Ewart.

†† Elfving. Ueber die Einwirkung von Aether u. Chloroform auf die Pflanzen. Oefversigt af Finska Vetensk. Soc. Forh. **28**: 36. 1886.

‡‡ Laurén. Bot. Jahresb. **20**[1]: 92. 1892.

§§ Johanssen, W. L. Das Aether-Verfahren beim Frühtreiben. Jena. 1900.

|||| Bot. Gaz. *l. c.*

¶¶ *L. c.*

*** *L. c.*

to a lesser extent were grown on the nutrient solutions prescribed by Pfeffer * and designated as A and C in the first paper on the subject by Richards,† the cultures confined in an atmosphere of chloroform of known or approximately known value and the effect upon dry weight of the crop, economic coefficient and acid coefficient noted.

The work was done in the botanical laboratory of Barnard College during the years 1904 and 1905 under the guidance of Dr. Herbert Maule Richards, to whom his student is much indebted for his kindness and interest. To the New York Botanical Garden many thanks are due for extending the privileges of the library.

To grow the fungi, small dishes 70 mm. in diameter and 30 mm. deep were used. These, in all series following IV, were of the non-sol glass of Whitall, Tatum & Co. Each culture dish rested on two strips of glass over a half Petri dish containing 40 c.c. of a 10 per cent. KOH solution. The Petri dish in turn sat upon a ground-glass plate. On glass strips laid across the culture dish was supported a small watch glass to receive the chloroform. In operating, each dish was arranged as described and all covered by a bell-jar which was ground down on to the glass plate in a glycerine-gelatine seal. After the seal had hardened, amounts of chloroform calculated to the capacity of the jars — due allowance having been made for the solids and liquids within — were run in through the neck which was at once closed with a rubber stopper. It is possible in this way to regulate the supply of chloroform vapor with tolerable accuracy, for the placing of the stopper in position occupies but a moment and relatively little of the chloroform can volatilize and escape. Moreover, the seal at the base of the jar is sufficiently strong to be proof against any small pressure generated by the vaporizing liquid.

In manipulation, the usual precautions were observed. The culture dishes, before each using, were soaked in dichromate cleaning solution, washed, rinsed in distilled and redistilled water and sterilized in hot air at 100°–120° C. The bell-jars were

* Pfeffer, W. Ueber Election organischer Nährstoffe. Jahrb. Wiss. Bot. **28**: 205. 1895.

† *L. c.*

rinsed in formalin ; the glass strips and watch glasses were washed and sterilized in hot air (100°–120° C.). All flasks and pipettes were handled similarly.

The redistilled water mentioned above was the distilled water furnished for ordinary laboratory purposes in the college — the still is tin-lined — redistilled and condensed through a block-tin tube. A considerable portion passing over at first was discarded and the operation stopped while a quantity remained in the distilling flask. This water was used in making all solutions, as well as for the final rinsings of the apparatus. Its specific conductivity as determined in the physics laboratory varied between 2.74×10^{-6} and 1.59×10^{-6}. The salts used were the best quality supplied by Merck & Co. White rock-candy furnished the sugar. A solution of 30–50 per cent. was made, filtered through glass wool to remove the bits of string and kept tightly corked for use. The proper amount from the stock was taken for each solution made for a series. Iron, also, was kept in solution, but the other salts were weighed out as required. The formulae of the solutions are as follows :

A		C	
NH_4NO_3	1.00 gm.	Asparagine	0.50 gm.
K_2HPO_4	0.50 gm.	KH_2PO_4	0.50 gm.
$MgSO_4$	0.25 gm.	$MgSO_4$	0.25 gm.
$FeSO_4$	trace.	$FeSO_4$	trace.
Sugar	5.00 gm.	Sugar	5.00 gm.
Water	100.00 c.c.	Water	100.00 c.c.

The chloroform was the best grade made by Merck, known to contain .02 per cent. of absolute alcohol to prevent decomposition, which, however, in the quantities of the anaesthetic used is negligible. Repeated tests with $AgNO_3$ showed that no free Cl was present.

Sowing the fungus was accomplished by dipping the end of a clean glass rod into the nutrient solution, rubbing it over the dry spores, redipping it into the fluid, and agitating well to secure uniform distribution. The smooth white felts of the control cultures testified to the effectiveness of the treatment and the purity of the reagents. In practice, enough nutrient solution was made at a time for one complete series — the iron alone omitted. This solution was boiled, cooled and 50 c.c. taken in an accurate pi-

pette to be analyzed for acid and sugar. The remainder was then inoculated in bulk with spores in the method described and doses of 50 c.c. run from the pipette into each culture dish. Last of all the iron, a mere trace, was added.

The crop was reaped when the controls showed a full fruited surface. The felts were thrown on weighed filters, washed thoroughly with boiling water, dried in hot air at 70°–80° C., cooled in a desiccator and the dry weight ascertained. The filtrate made up to 250 c.c. was analyzed, as recorded, for oxalic acid, or sugar, or both. Determinations for oxalic acid in the filtrate from the *Sterigmatocystis* * were made at once. Ten cubic centimeters of the solution were titrated against standardized NaOH. The total acidity, less the acidity at the time of setting up (that of the stock solution), gave the correct amount of acid produced during growth.

A measured quantity of the filtrate, generally 100 c.c., was next inverted by boiling with 0.5 c.c. of 5N HCl and the invert sugar estimated by the Fehling test. When asparagine was present, extra care was taken. A cubic centimeter or 1.5 c.c. of acid was used for inversion and the time of boiling extended, water being added if necessary. Asparagine, by virtue of its amide groups, will reduce a copper solution and invalidate a test for sugar in its presence. Hard boiling with HCl serves to destroy the molecule with the production of NH_4Cl, which does not interfere with the analysis. The Fehling solution was kept in two portions. The stock $CuSO_4$ contained 69.28 gms. per liter; the alkaline tartrate, 100 gms. of NaOH and 350 gms. of $KNaC_4H_4O_6$ per liter. Standardization was made against accurately freshly prepared 1 per cent. dextrose. For each analysis 5 c.c. of each stock solution and 40 c.c. of H_2O were used. Calculations were made on the basis that under such conditions 0.5 gm. of invert sugar reduces 97 c.c. of the Fehling solution.†

The fruiting of the control cultures, as mentioned, determined the reaping of the crop. The period of growth varied with the temperature. Near its optimum of 34° C., *Sterigmatocystis* was

* Wehmer. Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. Bot. Zeit. **49**: 1891.

† Sutton. Volumetric Analysis. Ed. 1904.

ready to harvest in seven to ten days when grown on solution A, or in four to five days when asparagine replaced the NH_4NO_3; while the period of cultivation extended over two to three weeks if the temperature fell to 20°–25° C. The lack of constant conditions is to be regretted, especially in view of indications which may be discussed later.

The first effect of $CHCl_3$ used in such an amount as is necessary to cause noticeable stimulation is to retard the germination of the spores. With the smallest quantities employed, the first hyphae did not appear until six to twenty-four hours after growth had started in the chloroform-free air. The second effect seen is the production of toughened, buckled felts by a rapid growth accompanied by repression of conidial fructification, as observed also when the organism is stimulated by salts, etc. At harvesting, felts grown under optimum stimulation gave on an average crops 200 per cent. to 300 per cent. as heavy as the normal, rising in some instances to 500 per cent. and 700 per cent. The curve representing the influence of $CHCl_3$ on growth is similar to those of other stimulants; it rises in a characteristic manner to a maximum and then falls again.

Sterigmatocystis cultivated on solution A at a temperature varying between 20° and 30° C. grows more luxuriantly when the content of the atmosphere is, per liter, the vapor of $\frac{1}{3}$ c.c. of liquid $CHCl_3$ and the beneficial effect increases until $\frac{1}{2}$–$\frac{7}{12}$ c.c. per liter are used, when decrease begins. If the bell-jar contains per liter over 1 c.c. of $CHCl_3$ (vaporized), germination is permanently stopped, although the spores will grow readily if placed in air. When asparagine, instead of NH_4NO_3, is the source of nitrogen (solution C) the sensitivity of the *Sterigmatocystis* is increased. Optimum growth then takes place when $\frac{1}{6}$–$\frac{1}{4}$ c.c. of $CHCl_3$ is present per liter, and $\frac{2}{3}$ c.c. is sufficient to arrest development.

The augmentation in weight is accompanied by a relatively lessened acid formation and sugar consumption; that is to say, the ratio of the acid to the crop, and of the sugar used to the crop is lowered. For example, in Series X, Table I, the acid coefficient is lowered from 2.3186 to .4160 grams per gram fungus when the atmosphere held $\frac{1}{2}$ c.c. of $CHCl_3$ vaporized and to .3551 grams per gram fungus when the content was $\frac{7}{12}$ c.c.; at the same time,

the economic coefficient fell from 11.493 to 3.214 and 3.201 grams per gram fungus respectively. The average figures for the atmospheres quoted are for acid coefficient 0.6932 and 0.5696 and for sugar 3.744 and 3.514 against 1.4123 and 5.358 for the normal culture. The economic coefficient of the controls varies considerably from that found by Kunstmann * and Richards,† to be sure, but the figures obtained are fairly stable. The same thing is true of the data for oxalic acid as compared with those of Ono.‡ That irregular fluctuations may be regarded as due to individual peculiarities is indicated by the consistency in the various factors. For instance, a lack of due increase in weight is, in general, accompanied by an abnormal rise in the coefficients of acid and sugar, pointing to internal differences rather than to errors in calculation or manipulation.

Chloroform belongs to the so-called catalytics in Loew's § classification. The small amount absorbed by the water of the nutrient solution affects the protoplasm so as to cause it to work more economically, possibly through action on the enzyme formed.|| However that may be, the figures point again to the explanation of the phenomena advanced by Richards¶ and by Ono** as his third alternative, *i. e.*, that the fungus can under the conditions produced by the stimulant thrive more economically.

The sensitivity of *Penicillium* on solution A is about equal to that of *Sterigmatocystis* on solution C.

Another question of interest is the stage in the growth at which the $CHCl_3$ is most potent. To learn this, series were run with the optimum of $CHCl_3$ placed in some when set up as before and in others after the spores had been allowed to start. The former were more prosperous (Table III); that is, the impulse given at germination is more effective than one applied later, but even after complete germination, the fungus responds, although in a lesser degree, in the characteristic fashion.

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* *L. c.*

† *L. c.*

‡ *L. c.*

§ Loew. "Naturliche System der Gift Wirkungen." Munich, 1893, as quoted by Davenport, Experimental Morphology, p. 7.

|| Hueppe (*l. c.*) suggests this vaguely.

¶ *L. c.*

** *L. c.*

That chloroform cannot furnish carbon was settled by sowing spores on a solution of the necessary salts, but omitting sugar, and then placing in optimum chloroform conditions. Not a sign of a germ tube appeared even after two weeks' time.

Although there are no direct data on this point at present, still it is very evident to the worker that a given amount of chloroform is more efficacious at a higher than at a lower temperature, that the optimum point recedes as the temperature rises. It was impossible to keep the temperature of the room in which the cultures were grown constant for any considerable period, but the truth of the statement was many times manifest at various stages in the development. In one or two instances when the crop ripened at from 32–34° C., the optimum was pushed back from $\frac{1}{2}$–$\frac{7}{12}$ to $\frac{5}{12}$ or even $\frac{1}{3}$ c.c. per litre.

The facts indicated are, that:

1. When present in small quantities $CHCl_3$ vapor acts as a characteristic stimulant to the growth of *Sterigmatocystis nigra* and *Penicillium glaucum*.

2. Larger quantities are inimical or fatal.

3. Increased growth is attended by relatively less acid formation and less sugar consumption indicating greater economy in metabolism.

4. The time of greatest sensitiveness is at the germination of the spores.

5. $CHCl_3$ acts as a stimulant purely since it cannot be a source of carbon.

6. The effect of a given amount of the anaesthetic is greater as the temperature rises.

BARNARD COLLEGE, COLUMBIA UNIVERSITY,
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TABLE I.

Sterigmatocystis nigra.—SOLUTION A.

	$CHCl_3$. per li.	Net Weight.	Per Cent. Increase Over Normal.	Oxalic Acid by Weight	Oxalic Acid per gm Fungus.	Sugar Used.	Economic Coefficient.	
							Sugar: Fungus.	Fungus: Sugar.
	c.c.	grams.		grams.	grams.	grams.		
Series I.	0	.5836	—	.3942	.6754	2.7069	4.6378	.2156
Time 14 days.	0	.6628	—	.4097	.6181	3.0790	4.6454	.2152
Temp. 26°–33°C.	$\frac{1}{3}$	.7400	18.74	.2164	.2924	2.1130	2.8554	.3502
10% sugar sol.	$\frac{5}{12}$	.7360	18.10	.1597	.2170	2.2754	2.9820	.3353
	$\frac{1}{2}$	.6838	9.72	.2860	.4182	1.9804	2.8960	.3453
	$\frac{7}{12}$	.7088	13.73	.1468	.2071	1.9885	2.8054	.3564
Series II.	0	.1850	—	.1933	1.044	.7926	4.284	.2334
Time 12 days.	0	.2128	—	.1571	.7382	.8888	4.176	.2394
Temp. 24°–27°C.	$\frac{5}{12}$	.2950	48.31	.3622	1.227	1.3099	4.437	.2254
	$\frac{1}{2}$	.3314	66.62	.2566	.7743	1.5031	5.435	.2204
	$\frac{7}{12}$	.4216	111.9	.2203	.5225	1.6829	3.991	.2505
	$\frac{2}{3}$	.3110	56.36	.1764	.5672	1.3443	4.321	.2314
Series III.	0	.1806	—	.1996	1.105	.7502	4.164	.2407
Time 12 days.	0	.1294	—	.1585	1.224	.5492	4.487	.2228
Temp. 24°–27°C.	$\frac{5}{12}$	.5036	224.9	.1558	.3093	1.5577	3.091	.3309
	$\frac{1}{2}$	.4530	192.2	.1276	.2816	1.3916	3.072	.3255
	$\frac{7}{12}$	.2448	57.93	.0866	.3537	.6952	2.839	.3521
	$\frac{2}{3}$	.1598	3.10	.0618	.3867	.4482	2.804	.4488
Series IV.	0	.1750	—	.1392	.7954	.639	3.651	.2738
Time 12 days.	0	.0790	—	.0928	1.174	.281	3.557	.2811
Temp. 22°–28°C.	$\frac{1}{3}$	.2620	106.3	.1624	.6198	1.084	4.137	.2147
	$\frac{5}{12}$	.2710	113.4	.1753	.6468	1.080	3.985	.2509
	$\frac{1}{2}$	.2100	65.4	.1469	.6995	.833	3.966	.2521
	$\frac{7}{12}$	.2780	118.9	.1469	.5284	1.178	4.237	.2359
	$\frac{2}{3}$	.2226	75.3	.1005	.4535	1.062	4.770	.2096
	$\frac{3}{4}$	.1690	33.1	.1237	.7319	.813	4.810	.2078
Series V.	0	.0920	—	.1391	1.512	.462	5.021	.1991
Time 9 days.	0	.0640	—	.1121	1.751	.353	5.515	.1813
Temp. 26°–31°C.	$\frac{1}{3}$	.1170	50.0	.2589	2.212	.595	5.085	.1966
	$\frac{5}{12}$	.1550	98.7	.2705	1.745	.747	4.819	.2075
	$\frac{1}{2}$	.1370	75.6	.2628	1.918	.704	5.138	.1946
	$\frac{7}{12}$	.1590	103.8	.2551	1.604	.767	4.824	.2073
	$\frac{2}{3}$	.1110	44.3	.2164	1.949	.630	5.676	.1761
	$\frac{3}{4}$	.1750	124.3	.2056	1.174	.837	4.783	.2090
Series VI.	0	.0564	—	.0773	1.371	0.181	3.195	.3130
Time 7 days.	0	.0360	—	.0619	1.719	0.141	3.917	.2553
Temp. 27°–34°C.	$\frac{1}{3}$	.3650	690.0	.2783	0.762	1.480	4.055	.2466
	$\frac{5}{12}$	.2020	337.2	.1739	0.861	0.750	3.713	.2693
	$\frac{1}{2}$	.2720	488.7	.1662	0.611	0.623	2.290	.4366
	$\frac{7}{12}$	.2736	492.2	.1971	0.724	0.976	3.567	.2803
	$\frac{2}{3}$	.2256	388.3	.1469	0.651	0.623	2.761	.3621
	$\frac{3}{4}$	.2246	386.1	.1469	0.654	0.701	3.121	.3204

TABLE I.—*Concluded.*

Sterigmatocystis nigra.—SOLUTION A.

	$CHCl_3$. per li.	Net Weight.	Per Cent. Increase Over Normal.	Oxalic Acid by Weight.	Oxalic Acid per gm. Fungus.	Sugar Used.	Economic Coefficient.	
							Sugar: Fungus.	Fungus: Sugar.
	c.c.	grams.		grams.	grams.	grams.		
Series VII.	0	.0200	—	.0541	2.705	.253	12.64	.0791
Time 10 days.	0	.0280	—	.0541	1.932	.290	10.35	.0967
Temp. 24°–30°C.	$\frac{1}{3}$	.1290	437.5	.1852	1.436	.574	4.453	.2246
	$\frac{5}{12}$	.1340	458.3	.0696	0.5194	.518	3.865	.2587
	$\frac{1}{2}$	.1952	713.3	.0812	0.4160	.627	3.214	.3111
	$\frac{7}{12}$	.1960	716.7	.0696	0.3551	.627	3.201	.3124
	$\frac{2}{3}$	.1854	672.5	.0696	0.3754	.653	3.520	.2841
	$\frac{3}{4}$	.1420	491.7	.0501	0.3528	.326	2.296	.4356
Series VIII.	0	.0570	—	.1314	2.305	.343	6.025	.1659
Time 13 days.	0	.0660	—	.1275	1.932	.361	5.462	.1830
Temp. 24°–30°C.	$\frac{1}{3}$	.0980	59.4	.1469	1.499	.488	4.984	.2006
	$\frac{5}{12}$	.1990	223.6	.0696	0.3497	.574	2.885	.3387
	$\frac{1}{2}$	.1810	194.3	.0773	0.4271	.713	3.940	.2538
	$\frac{7}{12}$	.2650	330.9	.0696	0.2626	.701	2.647	.3778
	$\frac{2}{3}$	.1880	205.7	.0503	0.2676	.427	2.287	.4408
	$\frac{3}{4}$	.1130	83.7	.0194	0.1717	.253	2.237	.4470
Series IX.	0	.0820	—					
Time 21 days.	0	.0987	—					
Temp. 23°–25°C.	$\frac{1}{3}$	.1810	101.3					
	$\frac{5}{12}$	.1930	114.7					
	$\frac{1}{2}$	.2508	178.9					
	$\frac{7}{12}$	.1510	68.0					
	$\frac{2}{3}$	.3594	299.8					
	$\frac{3}{4}$	.2390	165.9					

TABLE II.

AVERAGES OF DATA GIVEN IN TABLE I.

$CHCl_3$ per li.	Net Weight.	Per Cent. Increase Over Normal.	Oxalic Acid by Weight.	Oxalic Acid per gm. Fungus.	Sugar Used.	Economic Coefficient.	
						Sugar: Fungus.	Fungus: Sugar.
c.c.	grams.		grams.	grams.	grams.		
0	—	—	0.1563	1.4123	0.754	5.3579	0.2122
$\frac{1}{3}$	0.2702	209.0	0.2080	1.1368	1.056	4.2615	0.2388
$\frac{5}{12}$	0.2987	181.9	0.1796	0.7344	1.101	3.722	0.2771
$\frac{1}{2}$	0.3015	220.5	0.1756	0.6932	1.047	3.744	0.2924
$\frac{7}{12}$	0.2998	223.7	0.1490	0.5696	1.077	3.514	0.2966
$\frac{2}{3}$	0.2518	249.3	0.1174	0.6643	0.741	3.734	0.3076
$\frac{3}{4}$	0.1771	214.1	0.1091	0.6168	0.586	3.449	0.3239

TABLE III.

Sterigmatocystis nigra.—SOLUTION C.

	$CHCl_3$ per li.	Net Weight.	Sugar Used.	Economic Coefficient.	
				Sugar: Fungus.	Fungus: Sugar.
	c.c.	grams.	grams.		
Series X.	0	0.0596	1.167	19.58	0.0511
Time 7 days.	0	0.0340	1.167	34.32	0.0291
Temp. 25°–32° C.	$\frac{1}{12}$	0.0210	0.858	40.86	0.0245
	$\frac{1}{6}$	0 0290	0.858	29.59	0.0339
	$\frac{1}{4}$	0.1150	1.038	9.026	0.1108
	$\frac{1}{3}$	0.0720	0.930	12.91	0.0774
	$\frac{5}{12}$	0.0650	0.832	12.80	0.0781
	$\frac{1}{2}$	0.1120	0.930	8.303	0.1204
Series XI.	0	0.0886			
Time 7 days.	$\frac{1}{3}$	0.3106			
Temp. 30°–31° C.	$\frac{5}{12}$	0.3610			
	$\frac{1}{2}$	0.2340			
Series XII.	0	0.1910			
Time 4 days.	0	0.1660			
Temp. 32°–34° C.	$\frac{1}{6}$	0.2810			
	$\frac{1}{4}$	0.2860			
	$\frac{1}{3}$	0.1920			
Series XIII.	0	0.4470			
Time 4 days.	0	0.3520			
Temp. 27°–32° C.	$\frac{1}{12}$	0.2200			
	$\frac{1}{6}$	0.4660			
	$\frac{1}{4}$	0.5790			
	$\frac{1}{3}$	0.2130			
	$\frac{5}{12}$	0.2120			
	$\frac{1}{2}$	0.1980			

TABLE IV

Sterigmatocystis nigra.—SOLUTION A

	$CHCl_3$ per li.	Net Weight.	Oxalic Acid by Weight.	Oxalic Acid per Gm. Fungus.	Sugar Used.	Economic Coefficient.	
						Sugar: Fungus.	Fungus: Sugar.
Series XIV.	c.c.	grams.	grams.	grams.	grams.		
Temp. 24°–31° C.	0	0.1470	0.1276	0.8680	0.477	3.245	0.3082
$CHCl_3$ in before germination, out after germination, *i. e.*, in 7 days. Total time 9 days.	0	0.0950	0.1044	1.099	0.274	2.884	0.3467
	$\frac{1}{2}$	0.3200	0.1121	0.3503	0.899	2.809	0.3559
	$\frac{7}{12}$	0.1500	0.0348	0.2320	0.365	3.063	0.4110
$CHCl_3$ after germination, *i. e.*, after 2 days; $CHCl_3$ in 10 days. Total time 12 days.	0	0.0840	0.1585	1.887	0.441	5.250	0.1905
	0	0.0794	0.1778	2.239	0.365	4.597	0.2175
	$\frac{1}{2}$	0.1240	0.0309	0.2492	0.404	3.258	0.3069
	$\frac{7}{12}$	0.2530	0.0619	0.2447	0.640	2.530	0.3953
Series XV.							
Temp 27°–32° C.	0	0.2200	0.1925	0.8750	0.957	4.350	0.2299
$CHCl_3$ in before germination, out after germination, *i. e.*, in 3 days. Total time 7 days.	0	0.1336	0.2040	1.527	0.777	5.816	0.1719
	$\frac{1}{2}$	0.2760	0.1584	0.5739	1.015	3.677	0.2719
	$\frac{7}{12}$	0.3040	0.1198	0.3941	0.996	3.431	0.2915
$CHCl_3$ in after germination, *i. e.*, after 2 days; $CHCl_3$ in 8 days. Total time 10 days.	0	0.1780	0.2744	1.542	0.936	5.258	0.1902
	0	0.1350	0.2435	1.804	0.751	5.563	0.1798
	$\frac{1}{2}$	0.0490	0.0154	0.3143	0.257	5.245	0.1907
	$\frac{7}{12}$	0.0280	0.0193	0.6893	0.257	9.178	0.1089
Series XVI.							
Temp. 23°–29° C.	0	0.0780					
$CHCl_3$ in before germination, in 4 days. Total time 14 days.	$\frac{1}{2}$	0.3730					
	$\frac{7}{12}$	0.1700					
$CHCl_3$ in after germination. In 14 days. Total time 16 days.	0	0.1190					
	$\frac{1}{2}$	0.2000					
	$\frac{7}{12}$	0.2676					
Series XVII.							
Temp. 26°–28° C.	0	0.0674					
$CHCl_3$ in before germination. In 4 days. Total time 8 days.	$\frac{1}{2}$	0.1470					
	$\frac{7}{12}$	0.1530					
$CHCl_3$ in after germination. In 12 days. Total time 12 days.	0	0.1040					
	$\frac{1}{2}$	0.0330					
	$\frac{7}{12}$	0.1040					

TABLE V

Penicillium glaucum.—SOLUTION A

	$CHCl_3$ per li.	Net Weight.	Sugar Used.	Economic Coefficient.	
				Sugar : Fungus.	Fungus : Sugar.
	c.c.	grams.	grams.		
Series XVIII.	0	0.0330	0.288	8.727	0.1145
Time 17 days.	0	0.0620	0.397	6.403	0.1561
Temp. 18°–26° C.	$\frac{1}{2}$	0.1320	0.726	5.500	0.1818
	$\frac{1}{6}$	0.1124	0.532	4.733	0.2112
	$\frac{1}{4}$	0.1054			
	$\frac{1}{8}$	0.0950	0.523	5.505	0.1816
	$\frac{5}{12}$	0.0540	0.302	5.592	0.1788
	$\frac{1}{2}$	0.0416	0.257	6.178	0.1618
Series XIX.	0	0.0664			
Time 14 days.	0	0.0930			
Temp. 19°–24° C.	$\frac{1}{12}$	0.0700			
	$\frac{1}{6}$	0.1580			
	$\frac{1}{4}$	0.2980			
	$\frac{1}{8}$	0.0400			
	$\frac{5}{12}$	0.0760			
	$\frac{1}{2}$	0.0400			
Series XX.	0	0.0490			
Time 19 days.	0	0.0534			
Temp. 21°–26° C.	$\frac{1}{12}$	0.0720			
	$\frac{1}{6}$	0.0950			
	$\frac{1}{4}$	0.0580			
	$\frac{1}{8}$	0.0268			
	$\frac{5}{12}$	0.0570			
	$\frac{1}{2}$	0.0300			